REMARKS

Applicants have incorporated the limitations recited in claims 14 and 19 into claims 4 and 1, respectively, and cancelled claims 14 and 19. Note that claims 2, 3, 10-3, 15, and 17 were cancelled in the previously filed response to the first office action.

Claims 1, 4-9, 16, 18, and 20-24 are currently pending. Reconsideration of this application, as amended, is respectfully requested in view of the remarks below.

Rejection under 35 U.S.C. 112, second paragraph

Claims 1, 4-9, 14, 16, and 18-20 are rejected for being indefinite. Claims 14 and 19 have been cancelled.

The Examiner asserts that claims 1 and 4 fail to recite numerical values for each quartile necessary for assessing aspirin and relative risk of a cardiovascular event. See the Office Action, page 2, lines 11-21. Applicants have amended both claims to recite "wherein the first quartile comprises concentrations less than 15.1 ng/mmol creatinine, the second quartile comprises concentrations between 15.1 ng/mmol creatinine and 21.8 ng/mmol creatinine, the third quartile comprises concentrations between 21.9 ng/mmol creatinine and 33.7 ng/mmol creatinine, and the fourth quartile comprises concentrations greater than 33.8 ng/mmol creatinine."

The Examiner also asserts that the term "predetermined set of concentration quartiles" recited in claims 1 and 4 is not defined. See the Office Action, page 3, lines 1-3. The just-mentioned amendment has rendered moot this ground for rejection.

Claims 5-9, 16, 18, and 20 depend from claim 1 or 4. It appears that the Examiner rejects them on the basis that their parent claims are indefinite. In view of the amendment to claims 1 and 4, Applicants believe that the rejection has been overcome.

Rejection under 35 U.S.C. 112, first paragraph

The Examiner rejects claims 14, 16, 19, and 21-24, alleging that they do not comply with the enablement requirement. Claims 14 and 19 have been cancelled and the limitations recited

therein have been incorporated into claims 4 and 1, respectively. Claims 1 and 4 will therefore be discussed instead.

Claim 1 covers a method of assessing aspirin resistance. Claims 4, 16, and 21-24 cover a method of assessing risk of a cardiovascular event. Each of claims 1, 4, and 16 requires using a predetermined set of quartiles each having a specific concentration range. Each of claims 21-24 requires using a specific predetermined comparison concentration.

The Examiner asserts that a person of ordinary skill, in view of the specification, would not be enabled to used the claimed methods. More specifically, he points out that the data presented in Table 3 of the specification (on which the specific concentration ranges and concentration mentioned in the above paragraph are based on) shows a high degree of overlap between the four quartiles and some p values greater than 0.05.[1] He proceeds to conclude that a person skilled in the art would reject "the proposition that there is a correlation between aspirin resistance and 11-dehydro thromboxane B2 concentrations." See the Office Action, page 4, lines 2-7.

Clearly, the Examiner is of the opinion that the asserted utility is incredible. In order words, he believes that this invention lacks utility. According to MPEP, 2164.07 I(A), "Office personnel should not impose a 35 U.S.C. 112, first paragraph, rejection grounded on a 'lack of utility' basis unless a 35 U.S.C. 101 rejection is proper." Here, a rejection under 35 U.S.C. 101 is clearly proper. However, Applicants disagree with the Examiner that the data shown in Table 3 is not credible.

Table 3 of this application shows that a person, who has a high urinary concentration of 11-dehydro thromboxane B2 after taking aspirin, is statistically at a higher risk of a cardiovascular event, compared to one who has a low concentration after taking aspirin. In Table 3, the p values are in the range of <0.001 to 0.34 and there is an overlap in 95% confidence intervals between two adjacent quartiles. Applicants would like to point out that it is acceptable in the field of prognosing a cardiovascular event that data showing a trend do not necessarily meet high statistical standards. Applicants have attached hereto as "Exhibit A" Eikelboom et al.,

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[1] The Examiner inadvaertantly wrote in the office action that "the p values ... appears to be less than the significant level of $\alpha = 0.05$."

Circulation, 2002: 1652, a paper by the lead inventor's group. This paper describes the claimed subject matter. Indeed, Table 3 thereof includes the same data presented in Table 3 of the instant specification. This paper has been cited as many as 31 times since it was published about 3 years ago. To the best knowledge and belief of Applicants' counsel, these 31 citations are strong evidence that the data has been widely accepted. Attached as "Exhibit B" is a copy of a HTTP version of this paper and a list of the 31 articles citing it (see pages 13-19).

According to MPEP 2164.07. I(C), "evidence will be sufficient if, considered as a whole, it leads to a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true." In view of the facts set forth above, Applicants submit that a skilled person would accept a correlation between the aspirin resistance/risk of a cardiovascular event and the 11-dehydro thromboxane B2 concentrations.

The Examiner further provides a list of characteristics needed to be considered in developing a suitable diagnostic assay, citing Strongin (1993, "Sensitivity, specificity and predictive value of diagnostic tests"). Applicants would like to point out that these characteristics are criteria for examining a method of diagnosing of a disease. The standard for such an examination is high, as diagnosis is vital in treating or curing a disease which a patient is currently suffering from. The present invention is a method of assessing the likelihood that a person will have a cardiovascular event in the future so that he can take precautions. Thus, it does not need to comply with the high standard adopted in Strongin. Applicants have attached as "Exhibit C" a copy of a report published on News-Medical.Net (http://www.news-medical.net/?id=6156). According to the report, this invention is useful in prognosing, not diagnosing, cardiovascular disease

In sum, contrary to the Examiner's assertion, a skilled person in the art, in view of the specification, would conclude that the asserted utility is credible. Applicants respectively request that this rejection be withdrawn.

Rejection under 35 U.S.C. 103(a)

The Examiner rejects claims 1, 4-9, 14, 16, and 18-24 for obviousness, relying on Ens, WO 01/31052 (Ens), Cipollone et al. 102 Circulation (2000) 1007 (Cipollone), and Armitage et al., Encyclopedia of Biostatics (1998) (Armitage). Claims 14 and 19 have been cancelled. Claims 1, 4, and 20, the independent claims, will be discussed first.

Claims 1 and 4, as amended, cover methods for assessing aspirin resistance and determining risk of a cardiovascular event, respectively. Each method includes (1) determining the concentration of a thromboxane A2 metabolite in a sample from the patient, (2) comparing the concentration to a predetermined set of first, second, third, and fourth quartiles, and (3) determining within which quartile the sample concentration falls. Aspirin resistance or risk of a cardiovascular event increases as the thromboxane A2 metabolite concentration falls within from the first to the second, to the third, and to the fourth quartile. The first, second, third, and fourth quartiles contain thromboxane A2 metabolite concentrations corresponding to less than 15.1 ng/mmol creatinine, 15.1-21.8 ng/mmol creatinine, 21.9-33.7 ng/mmol creatinine, and greater than 33.8 ng/mmol creatinine, respectively.

Ens discloses a method for identifying a minimal aspirin dose for platelet inhibition based on measuring the concentration of thromboxane B2, a thromboxane A2 metabolite. It merely teaches a thromboxane B2 concentration above which aspirin resistance is considered a possibility. See page 11, lines 15-19. It does not suggest that the degree of aspirin resistance and risk of a cardiovascular event increase as the thromboxane B2 concentration increases. Thus, it does not suggest comparing a set of quartiles having increasing thromboxane A2 metabolite concentration ranges, let alone the specific concentration ranges of a thromboxane A2 metabolite recited in claims 1 and 4.

Cipollone and Armitage do not cure this decificiency. Cipollone teaches a normal range of 17.0-28.3 ng/mmol of 11-dehydro-thromboxane B2 in patients taking aspirin. Armitage teaches using quartiles as a useful tool for modeling a risk relationship. Both references do not disclose or suggest that the degree of aspirin resistance and risk of a cardiovascular event increase as the concentration of thromboxane B2 increases.

As discussed above, none of Ens, Cipollone, and Armitage suggests a correlation between the concentration of a thromboxane A2 metabolite and aspirin resistance/risk of a cardiovascular event. Thus, a person skilled in the art, in view of the these three references, would not have arrived at the methods of claims 1 and 4, i.e., assessing aspirin resistance and risk of a cardiovascular event based on thromboxane A2 metabolite concentrations. In other words, claims 1 and 4 are not rendered obvious by Ens, Cipollone, and Armitage.

Of note, the Examiner points out that "Ens discloses the division of data into 11 different [thromboxane B2] concentration quartiles (see table 2)." See the Office Action, page 5, lines 17-18. Table 2 of Ens shows that the "false" and "true" rates for positive aspirin resistance and negative aspirin resistance at 11 thromboxane B2 concentrations. Based on this data, Ens selects a thromboxane B2 concentration, i.e., 800 pg/mg, as a point above which aspirin resistance is deemed to have possibly taken place. See page 11, lines 15-19. The data in no way suggests a correlation between the thromboxane B2 concentration and the degree of aspirin resistance and risk of a cardiovascular event.

Applicants now turn to claim 21. Claim 21 covers a method for determining relative risk of a cardiovascular event by determining whether the concentration of 11-dehydro thromboxane B2 in a urine sample exceeds 15.1 ng/mmol. In this method, a 11-dehydro thromboxane B2 concentration greater than 15.1 ng/mmol is indicative of increased risk of a cardiovascular event. As discussed above, none of Ens, Cipollone, and Armitage discloses or suggests a correlation between the risk of a cardiovascular event and thromboxane B2 concentrations of a patient taking aspirin. It should be pointed out that Ens merely discloses monitoring the thromboxane B2 concentration in a patient to determine aspirin's antithrombotic efficacy, not to determine risk of a cardiovascular event. See Example 5, page 14, lines 5-17. Indeed, all three references are silent on determining risk of a cardiovascular event based on a thromboxane B2 concentration, let alone the specific concentration recited in claim 21. Thus, claim 21 is not rendered obvious by Ens, Cipollone, and Armitage.

Claims 5-9 and 16 depend from claim 4, claims 18 and 20 depend from claim 1, and claims 21-24 depend from claim 20. For the same reasons set forth above, these claims are also not obvious over the cited references.

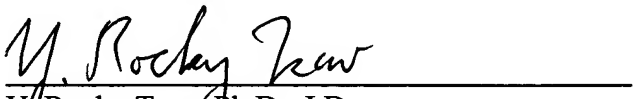
CONCLUSION

For the reasons set forth above, Applicants submit that the grounds for the objections and rejections asserted by the Examiner have been overcome and claims 1, 4-9, 16, 18, and 20-24, as pending, cover subject matter that is novel and unobvious over the prior art. Applicants request that all pending claims be allowed.

Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 5-9-05



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Aspirin-Resistant Thromboxane Biosynthesis and the Risk of Myocardial Infarction, Stroke, or Cardiovascular Death in Patients at High Risk for Cardiovascular Events

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Background—We studied whether aspirin resistance, defined as failure of suppression of thromboxane generation, increases the risk of cardiovascular events in a high-risk population.

Methods and Results—Baseline urine samples were obtained from 5529 Canadian patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study. Using a nested case-control design, we measured urinary 11-dehydro thromboxane B_2 levels, a marker of in vivo thromboxane generation, in 488 cases treated with aspirin who had myocardial infarction, stroke, or cardiovascular death during 5 years of follow-up and in 488 sex- and age-matched control subjects also receiving aspirin who did not have an event. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of 11-dehydro thromboxane B_2, with patients in the upper quartile having a 1.8-times-higher risk than those in the lower quartile (OR, 1.8; 95% CI, 1.2 to 2.7; $P=0.009$). Those in the upper quartile had a 2-times-higher risk of myocardial infarction (OR, 2.0; 95% CI, 1.2 to 3.4; $P=0.006$) and a 3.5-times-higher risk of cardiovascular death (OR, 3.5; 95% CI, 1.7 to 7.4; $P<0.001$) than those in the lower quartile.

Conclusions—In aspirin-treated patients, urinary concentrations of 11-dehydro thromboxane B_2 predict the future risk of myocardial infarction or cardiovascular death. These findings raise the possibility that elevated urinary 11-dehydro thromboxane B_2 levels identify patients who are relatively resistant to aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block in vivo thromboxane production or activity. (***Circulation.* 2002;105:1650-1655.**)

Key Words: aspirin ■ thromboxane ■ atherosclerosis ■ myocardial infarction ■ stroke

Aspirin reduces the risk of cardiovascular events by ≈25% in a broad category of patients with arterial vascular disease.[1] However, its effectiveness is limited because 10% to 20% of patients with arterial thrombosis who are treated with aspirin have a recurrent vascular event during long-term follow-up.[2] Aspirin exerts its major antithrombotic effect by irreversibly acetylating platelet cyclo-oxygenase-1, thereby inhibiting thromboxane A_2 synthesis. Although other poorly defined effects of aspirin on platelet function have been described, their contribution to the antithrombotic effect of aspirin is uncertain.[2,3]

See p 1620

There are several possible explanations for the limited efficacy of aspirin. First, it is well recognized that platelets can be activated by pathways that are not blocked by aspirin.[4–7] Second, it has been suggested that higher doses of aspirin than are currently used (75 to 325 mg/d) may be required in some patients to achieve the optimal antithrombotic effect of aspirin.[8,9] However, low-dose aspirin blocks > 95% of platelet cyclooxygenase-1 activity,[2,10] and there is no convincing evidence that the antithrombotic effect of aspirin is dose-related.[11–13] Third, some patients may be able to generate thromboxane A_2 despite usual therapeutic doses of aspirin and therefore fail to benefit from aspirin treatment.[10] The clinical importance of this third mechanism is unclear. All three potential causes of aspirin failure have been designated as aspirin resistance. For the purpose of the present study, we use the term to describe the third potential mechanism, namely, incomplete suppression of thromboxane generation with the usual dose of aspirin. The extent of inhibition of thromboxane A_2 generation can be determined by measuring urinary levels of 11-dehydro thromboxane B_2, a stable metabolite of thromboxane A_2.[14] Accordingly, base-

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line urinary 11-dehydro thromboxane B_2 concentrations were measured in 976 aspirin-treated patients at high risk of cardiovascular events from Canadian centers who were enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study to determine whether incomplete suppression of thromboxane generation is associated with an increased risk of recurrent cardiovascular events.

Methods

Patients

The HOPE study[15,16] was an international, randomized, placebo-controlled, 2×2 factorial trial of ramipril and vitamin E for the secondary prevention of cardiovascular disease. The institutional review committee at each participating center approved the study, and all subjects gave informed consent. A total of 9541 patients ≥55 years of age at the time of randomization who had a history of coronary artery disease, stroke, peripheral vascular disease, or diabetes plus at least one other cardiovascular risk factor were assigned to one of four treatments: ramipril titrated up to 10 mg daily, 400 IU vitamin E daily, both, or neither. The study commenced in December 1993 and was terminated prematurely on March 22, 1999, because of clear evidence of a benefit of ramipril.

Urine Sample Collection

All study participants were asked to provide a first morning urine specimen at the time of randomization. Of the 9541 patients in the HOPE study, 9282 (97%) provided baseline urine samples.[17] Samples (n=5529) from the 129 Canadian centers participating in this study were sent to the central laboratory in Hamilton, Canada, where they were stored at −80°C until analysis. Only samples from Canadian centers were used for the present study.

Follow-Up and Ascertainment of Clinical Outcomes

All patients in the HOPE study were followed at 1 month, 6 months, and 6-month intervals thereafter until completion of the study. At each follow-up, clinical outcomes were recorded and medication use, including aspirin, was documented. The primary outcome was the composite of myocardial infarction, stroke, and death from cardiovascular causes, as previously defined.[15,16]

Selection of Cases and Control Subjects

Of patients with available urine samples (n=5529), only those who were taking aspirin at the time of commencement of the run-in phase (before randomization), at randomization (coinciding with the time of urine collection), and at each follow-up visit were eligible for inclusion. Aspirin-treated patients who provided an adequate baseline sample of urine and had a confirmed myocardial infarction, stroke, or cardiovascular death after randomization were defined as cases. Control subjects were randomly selected from aspirin-treated patients who provided an adequate baseline urine sample but did not have myocardial infarction, stroke, or cardiovascular death after randomization. Control subjects were matched according to sex and age (±5 years) in a 1:1 ratio with cases.

Laboratory Analysis

For each case and control subject, urine collected and stored at baseline was thawed and assayed for 11-dehydro thromboxane B_2 levels with a commercially available enzyme immunoassay (Cayman Chemical) that has interassay and intra-assay coefficients of variation of 12.1% and 10%, respectively. Assays were performed by laboratory staff blinded to patient status as case or control subject. In addition, case and control specimens were assayed in random order, thereby reducing the possibility of systematic bias.

Statistical Analysis

Means or proportions for baseline demographics and risk factors were calculated for cases and control subjects. The significance of any difference between cases and control subjects was tested by means of Student's paired t test for means and McNemar χ^2 test for proportions, which takes into account the matching between cases and control subjects. Because 11-dehydro thromboxane B_2 values are skewed, geometric means were calculated after log transformation of the raw data; the significance of any differences in geometric mean values between cases and control subjects was tested by means of Student's paired t test. Median concentrations also were calculated, and levels in cases and control subjects were compared by means of Wilcoxon's rank-sum test.

Tests for trend were used to assess any association between increasing baseline urinary 11-dehydro thromboxane B_2 concentrations and risk of myocardial infarction, stroke, or cardiovascular death after dividing the samples into quartiles defined by the distribution of the complete cohort. Adjusted estimates of the association between increasing baseline urinary 11-dehydro thromboxane B_2 concentrations and risk of myocardial infarction, stroke, or cardiovascular death were obtained by means of conditional logistic regression modeling that accounted for the matching variables and controlled for the random treatment assignment and baseline differences between cases and control subjects. A separate multivariable regression model was used to examine the association between baseline patient characteristics, including age, sex, heart rate, blood pressure, body mass index, past history of vascular disease, conventional vascular risk factors, lipid-lowering therapy, β-blockers, diuretics, and randomized treatment allocation (ramipril or vitamin E) and urinary 11-deydro thromboxane B_2 concentrations in the urine.

All probability values are 2-sided; confidence intervals were calculated at the 95% level.

Results

Baseline characteristics of cases and control subjects are shown in Table 1. As expected, patients in whom myocardial infarction, stroke, or cardiovascular death subsequently developed had a higher mean body mass index and baseline blood pressure and were more likely than those who remained free of these events to be current smokers or have a history of hypertension, diabetes, myocardial infarction, or peripheral vascular disease. Cases also were more often treated with diuretics or calcium channel blockers at baseline and less often treated with lipid-lowering drugs or randomized to ramipril therapy. Because of the matching, the age and sex of cases and control subjects were similar.

Geometric mean and median urinary concentrations of 11-dehydro thromboxane B_2 at baseline were significantly higher among patients who had subsequent development of the composite outcome of myocardial infarction, stroke, or cardiovascular death compared with those who remained free of these events (Table 2). The difference between cases and control subjects was greatest in those who had a myocardial infarction (24.5 versus 20.9 ng/mmol creatinine, $P=0.003$) or died of a cardiovascular cause (25.6 versus 20.4 ng/mmol creatinine, $P<0.001$). Baseline urinary concentrations of 11-dehydro thromboxane B_2 were not significantly different between cases who had subsequent development of stroke and their matched control group (25.0 versus 27.4 ng/mmol creatinine, $P=0.47$).

The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of baseline urinary 11-dehydro thromboxane

TABLE 1. Baseline Characteristics of Study Participants*

Characteristic	Cases (n=488)	Controls (n=488)	P
Age, y	67.3±7.2	67.4±7.2	0.78
Female sex, n (%)	77 (15.8)	77 (15.8)	...
Body mass index, kg/m^2	27.8±4.1	26.9±3.7	<0.001
Heart rate, bpm	66.2±10.3	65.6±10.9	0.41
SBP, mm Hg	137.1±20.6	133.5±18.0	0.002
DBP, mm Hg	76.6±9.8	75.6±9.4	0.08
History of coronary disease, n (%)			
Any	469 (96.1)	464 (95.1)	0.54
MI	364 (74.6)	309 (63.4)	<0.001
Stable angina	355 (72.7)	336 (68.9)	0.19
Unstable angina	184 (37.7)	176 (36.1)	0.65
CABG	176 (36.1)	154 (31.6)	0.15
PCI	87 (17.8)	104 (21.3)	0.22
Stroke or TIA, n (%)	59 (12.1)	40 (8.2)	0.06
Peripheral vascular disease, n (%)	240 (49.2)	173 (35.5)	<0.001
Hypertension, n (%)	219 (44.9)	154 (31.6)	<0.001
Diabetes, n (%)	159 (32.6)	105 (21.5)	<0.001
Elevated total cholesterol, n (%)	279 (57.2)	310 (63.5)	0.38
Current cigarette smoking, n (%)	81 (16.6)	57 (11.7)	0.03
Medications, n (%)			
Aspirin	488 (100)	488 (100)	...
β-Blocker	241 (49.4)	235 (48.2)	0.76
Lipid-lowering agent	121 (24.8)	166 (34.0)	0.002
Diuretics	73 (15.0)	34 (7.0)	<0.001
Calcium channel blockers	289 (59.2)	238 (48.8)	0.002
Ramipril	227 (46.5)	274 (56.1)	0.003
Vitamin E	246 (50.4)	252 (51.6)	0.74

Values are mean±SD or n (%). CABG indicates coronary artery bypass graft surgery; CV, cardiovascular; DBP, diastolic blood pressure; MI, myocardial infarction; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; and TIA, transient ischemic attack.

B_2 concentration (P for trend across quartiles, 0.01), with patients in the highest quartile having a risk 1.8-fold higher than those in the lowest quartile (OR, 1.8; 95% CI, 1.2 to 2.9; P=0.009) (Figure). A similar association was seen with myocardial infarction (P for trend across quartiles, 0.005) and cardiovascular death (P for trend across quartiles, 0.001) but not for stroke (P for trend across quartiles, 0.20) (Table 3). Results were similar with or without adjustment for baseline differences between cases and control subjects, including conventional vascular risk factors, cointerventions, and randomized treatment allocation.

To evaluate whether increased baseline urinary 11-dehydro thromboxane B_2 concentrations were associated with early rather than late cardiovascular events, we performed separate analyses in patients who had an event within the first 12 months of study entry and those whose event occurred >12 months after study entry. The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death that was associated with the highest quartile of urinary 11-dehydro thromboxane B_2 as compared with the lowest quartile was 2.9 (95% CI, 0.9 to 9.1) for events occurring with the first 12 months and 1.7 (95% CI, 1.0 to 2.7) for events occurring after the first 12 months.

Using linear multivariable regression modeling, variables that were found to be independently associated with baseline urinary 11-dehydro thromboxane B_2 concentrations in the urine were female sex (P=0.004), body mass index (P=0.001), history of peripheral vascular disease (P=0.01), current cigarette smoking (P=0.09), use of calcium channel blockers (P=0.08), and randomization to vitamin E (P=0.04). However, these variables combined were able to predict <5% of the variation in urinary 11-dehydro thromboxane B_2 concentrations (R^2=0.045).

Discussion

This is the first study to demonstrate an association between aspirin resistance, defined as failure of suppression of thromboxane generation, and cardiovascular risk. In a well-defined cohort of aspirin-treated patients at high risk of cardiovascular events, increasing baseline urinary concentrations of 11-dehydro thromboxane B_2 were associated with an increasing risk of cardiovascular events, particularly myocardial infarction and cardiovascular death. This association was strong, graded, and independent of conventional vascular risk factors, including elevated body mass index, blood pressure,

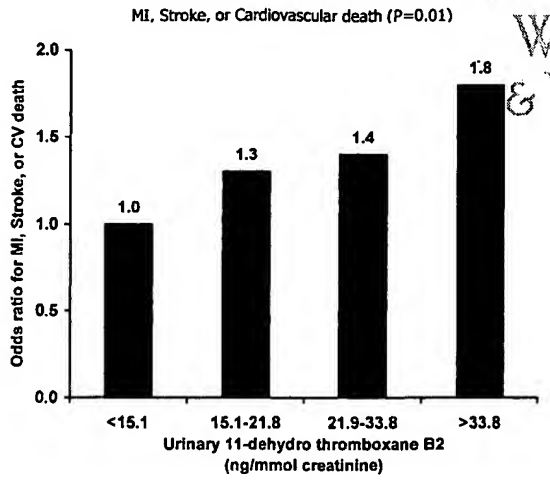
TABLE 2. Baseline Urinary Concentrations of Urinary 11-Dehydro Thromboxane B_2 in Cases and Controls

Outcome	11-Dehydro Thromboxane B_2 Concentration, ng/mmol Creatinine		
	Cases	Controls	*P*
MI, stroke, or CV death (n=488)			
Geometric mean	24.5	21.5	0.01
Median	22.7	21.0	0.01
MI (n=378)			
Geometric mean	24.5	20.9	0.003
Median	22.8	20.3	0.001
Stroke (n=80)			
Geometric mean	25.0	27.4	0.47
Median	21.3	25.9	0.40
CV death (n=244)			
Geometric mean	25.6	20.4	<0.001
Median	24.0	19.9	<0.001

MI indicates myocardial infarction; CV, cardiovascular.

hypertension, diabetes, smoking, and previous history of vascular disease. Moreover, the strength of the association was not modified by differences between cases and control subjects in the proportion of patients receiving lipid-lowering or antihypertensive therapy or by randomization to vitamin E or ACE inhibitors.

Several mechanisms can be proposed to account for the incomplete suppression of thromboxane generation by aspirin.[2] First, polymorphisms or mutations of the cyclooxygenase-1 gene that make it relatively resistant to inhibition by aspirin may provide a molecular basis for aspirin resistance. However, to our knowledge, such a mutation has not been identified. Second, nucleated cells such as monocytes or vascular endothelial cells can provide prostaglandin H_2 to platelets to bypass platelet cyclooxygenase-1 or can use prostaglandin H_2 to synthesize their own thromboxane A_2 because they are endowed with substantial amounts of thromboxane synthase.[4,18–20] Arachidonate conversion to prostaglandin H_2 is catalyzed by cyclooxygenase-1 or -2. Although low-dose aspirin permanently and completely blocks cyclooxygenase-1 in platelets, nucleated cells can regenerate the enzyme. Consequently, these cells can produce prostaglandin H_2 even in the face of aspirin treatment. In addition to cyclooxygenase-1–mediated prostaglandin H_2 generation, nucleated cells can also produce prostaglandin H_2 through cyclooxygenase-2.[20,21] Whereas cyclooxygenase-1 is blocked by 80 to 325 mg of aspirin, doses similar to that used in the HOPE trial, inhibition of cyclooxygenase-2 requires doses of aspirin in excess of 500 mg daily.[2] Unlike cyclooxygenase-1, which is constitutively expressed, cyclooxygenase-2 expression in nucleated cells is augmented 10- to 20-fold by inflammatory stimuli. Augmented cyclooxygenase-2 expression may contribute to aspirin resistance in patients with ischemic heart disease because atherosclerosis is an inflammatory disease.[21–23]



Association between quartiles of 11-dehydro thromboxane B_2 and composite of myocardial infarction (MI), stroke, or cardiovascular (CV) death after adjustment for baseline differences between cases and control subjects (*P* value is for trend of association).

Our finding of an independent albeit weak association between history of peripheral vascular disease and urinary 11-dehydro thromboxane B_2 levels in the urine is consistent with prior reports suggesting that the severity of atherosclerosis is an important determinant of thromboxane generation.[14,24] In patients being treated with aspirin, differences in the extent or severity of atherosclerosis are unlikely to affect de novo platelet thromboxane production because even very low doses of aspirin completely and irreversibly block platelet cyclooxygenase-1.[25] Upregulation of cyclooxygenase-2 has been demonstrated in atherosclerotic tissue[24] and may be associated with greater synthesis and transfer of prostaglandin H_2 to platelets, thereby bypassing platelet cyclooxygenase-1 and leading to aspirin-insensitive thromboxane biosynthesis in these patients. However, our study cannot distinguish between failure of suppression of platelet cyclooxygenase-1 and upregulation of COX-2 expression as the cause for the observed differences in 11-dehydro thromboxane B_2 excretion between cases and control subjects.

The reason for the lack of an association between urinary 11-dehydro thromboxane B_2 and risk of stroke is unclear. Aspirin reduces the risk of stroke in a broad category of high-risk patients[1,2]; elevated urinary concentrations of 11-dehydro thromboxane B_2 have been reported in patients after stroke,[26] and failure of aspirin to suppress "platelet reactivity" or inhibit platelet aggregation in response to various platelet agonists also has been documented in patients after stroke.[27–29] In our study, the mean urinary concentration of 11-dehydro thromboxane B_2 in cases who had a stroke was similar to that in all cases (25.0 versus 24.5 ng/mmol creatinine), but the corresponding urinary concentration in matched stroke control subjects with was higher than the concentration in all control subjects (27.4 versus 21.5 ng/mmol creatinine). However, the number of stroke cases and matched control subjects was relatively small (n=80). Given the clear and graded association between urinary 11-dehydro

TABLE 3. Adjusted Odds* of Future Cardiovascular Death, Myocardial Infarction, and Stroke According to Baseline Urinary Concentrations of 11-Dehydro Thromboxane B_2

	Quartiles of 11-Dehydro Thromboxane B_2 Concentration, ng/mmol Creatinine				
Outcome	<15.1	15.1–21.8	21.9–33.7	>33.7	*P*
MI/stroke/CV death (n=488)					
Odds ratio (95 CI)	1.0	1.3 (0.9–2.0)	1.4 (0.9–2.2)	1.8 (1.2–2.7)	0.01
P	...	0.13	0.09	0.009	
MI (n=378)					
Odds ratio (95 CI)	1.0	1.3 (0.8–2.1)	1.5 (1.0–2.5)	2.0 (1.2–3.4)	0.005
P	...	0.26	0.07	0.006	
Stroke (n=80)					
Odds ratio (95 CI)	1.0	2.5 (0.6–10.0)	0.6 (0.2–2.2)	0.6 (0.2–1.8)	0.20
P	...	0.18	0.45	0.34	
CV death (n=244)					
Odds ratio (95 CI)	1.0	2.0 (1.0–3.9)	2.5 (1.3–4.9)	3.5 (1.7–7.4)	0.001
P	...	0.06	0.006	<0.001	

*Adjusted for baseline differences between cases and controls.
MI indicates myocardial infarction; CV, cardiovascular.

thromboxane B_2 concentration and the composite outcome of myocardial infarction, stroke, or cardiovascular death, as well as other individual components of this outcome, the absence of a demonstrable association between stroke risk and urinary concentration of 11-dehydro thromboxane B_2 probably reflects a play of chance.

Our study has several potential limitations. First, there were important differences between cases and control subjects with regard to potentially important confounders, including body mass index, systolic blood pressure, hypertension, diabetes, smoking, history of vascular disease, and cointerventions. However, even after adjustment for these differences, a clear association between urinary 11-dehydro thromboxane B_2 concentrations and risk of death, myocardial infarction, and stroke was demonstrated. The weak association between baseline patient characteristics and urinary 11-dehydro thromboxane B_2 concentrations further supports the conclusion that confounding did not account for our results. Second, urinary 11-dehydro thromboxane B_2 concentrations may have been influenced by recent acute thrombotic events, such as myocardial infarction or stroke, processes that are known to be associated with platelet activation and enhanced urinary excretion of thromboxane metabolites. However, patients who had a myocardial infarction or stroke within the previous 7 weeks were not randomized into the HOPE study, making this explanation less likely. Third, single baseline determinations of urinary 11-dehydro thromboxane B_2 concentrations may not accurately reflect the extent of platelet activation over long periods of time. However, the association between elevated urinary 11-dehydro thromboxane B_2 concentrations at baseline and subsequent risk of cardiovascular events was evident both during the first 12 months after randomization and beyond 12 months, indicating a stable association over an extended period of time. Fourth, we did not confirm patient compliance with aspirin therapy by measuring salicylate levels in the blood or urine. However, we specifically assessed compliance with aspirin therapy at each follow-up visit and only considered patients for inclusion who were taking aspirin before randomization and at 6-month follow-up visits. Patients who discontinued aspirin at any time during the study were not included. Finally, the extent of biological variation in urinary 11-dehydro thromboxane B_2 levels is unknown but could potentially limit the value of this marker to predict the risk of future cardiovascular events in an individual patient.

We conclude that among aspirin-treated patients at high risk of cardiovascular events, persistent thromboxane generation predicts the risk of the composite outcome of myocardial infarction, stroke, or cardiovascular death, independent of other cardiovascular risk factors. These data raise the possibility that high urinary levels of 11-dehydro thromboxane B_2 can prospectively identify patients who are relatively resistant to conventional antithrombotic doses of aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block thromboxane production or activity.

Acknowledgments

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Clinical Investigation and Reports

Aspirin-Resistant Thromboxane Biosynthesis and the Risk of Myocardial Infarction, Stroke, or Cardiovascular Death in Patients at High Risk for Cardiovascular Events

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Abstract

Background— We studied whether aspirin resistance, defined as

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failure of suppression of thromboxane generation, increases the risk of cardiovascular events in a high-risk population.

Methods and Results— Baseline urine samples were obtained from 5529 Canadian patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study. Using a nested case-control design, we measured urinary 11-dehydro thromboxane B_2 levels, a marker of in vivo thromboxane generation, in 488 cases treated with aspirin who had myocardial infarction, stroke, or cardiovascular death during 5 years of follow-up and in 488 sex- and age-matched control subjects also receiving aspirin who did not have an event. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of 11-dehydro thromboxane B_2, with patients in the upper quartile having a 1.8-times-higher risk than those in the lower quartile (OR, 1.8; 95% CI, 1.2 to 2.7; P=0.009). Those in the upper quartile had a 2-times-higher risk of myocardial infarction (OR, 2.0; 95% CI, 1.2 to 3.4; P=0.006) and a 3.5-times-higher risk of cardiovascular death (OR, 3.5; 95% CI, 1.7 to 7.4; P<0.001) than those in the lower quartile.

Conclusions— In aspirin-treated patients, urinary concentrations of 11-dehydro thromboxane B_2 predict the future risk of myocardial infarction or cardiovascular death. These findings raise the possibility that elevated urinary 11-dehydro thromboxane B_2 levels identify patients who are relatively resistant to aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block in vivo thromboxane production or activity.

Key Words: aspirin • thromboxane • atherosclerosis • myocardial infarction • stroke

Introduction

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Aspirin reduces the risk of cardiovascular events by ≈25% in a broad category of patients with arterial vascular disease.[1] However, its effectiveness is limited because 10% to 20% of patients with arterial thrombosis who are treated with aspirin have a recurrent vascular event during long-term follow-up.[2] Aspirin exerts its major antithrombotic effect by irreversibly acetylating

platelet cyclo-oxygenase-1, thereby inhibiting thromboxane A_2 synthesis. Although other poorly defined effects of aspirin on platelet function have been described, their contribution to the antithrombotic effect of aspirin is uncertain.[2,3]

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There are several possible explanations for the limited efficacy of aspirin. First, it is well recognized that platelets can be activated by pathways that are not blocked by aspirin.[4–7] Second, it has been suggested that higher doses of aspirin than are currently used (75 to 325 mg/d) may be required in some patients to achieve the optimal antithrombotic effect of aspirin.[8,9] However, low-dose aspirin blocks > 95% of platelet cyclooxygenase-1 activity,[2,10] and there is no convincing evidence that the antithrombotic effect of aspirin is dose-related.[11–13] Third, some patients may be able to generate thromboxane A_2 despite usual therapeutic doses of aspirin and therefore fail to benefit from aspirin treatment.[10] The clinical importance of this third mechanism is unclear.

All three potential causes of aspirin failure have been designated as aspirin resistance. For the purpose of the present study, we use the term to describe the third potential mechanism, namely, incomplete suppression of thromboxane generation with the usual dose of aspirin. The extent of inhibition of thromboxane A_2 generation can be determined by measuring urinary levels of 11-dehydro thromboxane B_2, a stable metabolite of thromboxane A_2.[14] Accordingly, baseline urinary 11-dehydro thromboxane B_2 concentrations were measured in 976 aspirin-treated patients at high risk of cardiovascular events from Canadian centers who were enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study to determine whether incomplete suppression of thromboxane generation is associated with an increased risk of recurrent cardiovascular events.

Methods

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Patients

The HOPE study[15,16] was an international, randomized, placebo-controlled, 2x2 factorial trial of ramipril and vitamin E for the secondary prevention of cardiovascular disease. The institutional review committee at each participating center approved the study, and all subjects gave informed consent. A total of 9541

patients ≥55 years of age at the time of randomization who had a history of coronary artery disease, stroke, peripheral vascular disease, or diabetes plus at least one other cardiovascular risk factor were assigned to one of four treatments: ramipril titrated up to 10 mg daily, 400 IU vitamin E daily, both, or neither. The study commenced in December 1993 and was terminated prematurely on March 22, 1999, because of clear evidence of a benefit of ramipril.

Urine Sample Collection

All study participants were asked to provide a first morning urine specimen at the time of randomization. Of the 9541 patients in the HOPE study, 9282 (97%) provided baseline urine samples.[17] Samples (n=5529) from the 129 Canadian centers participating in this study were sent to the central laboratory in Hamilton, Canada, where they were stored at -80°C until analysis. Only samples from Canadian centers were used for the present study.

Follow-Up and Ascertainment of Clinical Outcomes

All patients in the HOPE study were followed at 1 month, 6 months, and 6-month intervals thereafter until completion of the study. At each follow-up, clinical outcomes were recorded and medication use, including aspirin, was documented. The primary outcome was the composite of myocardial infarction, stroke, and death from cardiovascular causes, as previously defined.[15,16]

Selection of Cases and Control Subjects

Of patients with available urine samples (n=5529), only those who were taking aspirin at the time of commencement of the run-in phase (before randomization), at randomization (coinciding with the time of urine collection), and at each follow-up visit were eligible for inclusion. Aspirin-treated patients who provided an adequate baseline sample of urine and had a confirmed myocardial infarction, stroke, or cardiovascular death after randomization were defined as cases. Control subjects were randomly selected from aspirin-treated patients who provided an adequate baseline urine sample but did not have myocardial infarction, stroke, or cardiovascular death after randomization. Control subjects were matched according to sex and age (±5 years) in a 1:1 ratio with cases.

Laboratory Analysis

For each case and control subject, urine collected and stored at baseline was thawed and assayed for 11-dehydro thromboxane B_2 levels with a commercially available enzyme immunoassay (Cayman Chemical) that has interassay and intra-assay coefficients of variation of 12.1% and 10%, respectively. Assays were

performed by laboratory staff blinded to patient status as case or control subject. In addition, case and control specimens were assayed in random order, thereby reducing the possibility of systematic bias.

Statistical Analysis

Means or proportions for baseline demographics and risk factors were calculated for cases and control subjects. The significance of any difference between cases and control subjects was tested by means of Student's paired *t* test for means and McNemar χ^2 test for proportions, which takes into account the matching between cases and control subjects. Because 11-dehydro thromboxane B_2 values are skewed, geometric means were calculated after log transformation of the raw data; the significance of any differences in geometric mean values between cases and control subjects was tested by means of Student's paired *t* test. Median concentrations also were calculated, and levels in cases and control subjects were compared by means of Wilcoxon's rank-sum test.

Tests for trend were used to assess any association between increasing baseline urinary 11-dehydro thromboxane B_2 concentrations and risk of myocardial infarction, stroke, or cardiovascular death after dividing the samples into quartiles defined by the distribution of the complete cohort. Adjusted estimates of the association between increasing baseline urinary 11-dehydro thromboxane B_2 concentrations and risk of myocardial infarction, stroke, or cardiovascular death were obtained by means of conditional logistic regression modeling that accounted for the matching variables and controlled for the random treatment assignment and baseline differences between cases and control subjects. A separate multivariable regression model was used to examine the association between baseline patient characteristics, including age, sex, heart rate, blood pressure, body mass index, past history of vascular disease, conventional vascular risk factors, lipid-lowering therapy, ß-blockers, diuretics, and randomized treatment allocation (ramipril or vitamin E) and urinary 11-deydro thromboxane B_2 concentrations in the urine.

All probability values are 2-sided; confidence intervals were calculated at the 95% level.

Results

Baseline characteristics of cases and control subjects are shown in Table 1. As expected, patients in whom myocardial

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infarction, stroke, or cardiovascular death subsequently developed had a higher mean body mass index and baseline blood pressure and were more likely than those who remained free of these events to be current smokers or have a history of hypertension, diabetes, myocardial infarction, or peripheral vascular disease. Cases also were more often treated with diuretics or calcium channel blockers at baseline and less often treated with lipid-lowering drugs or randomized to ramipril therapy. Because of the matching, the age and sex of cases and control subjects were similar.

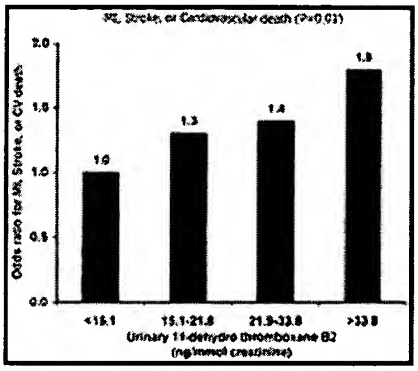
View this table: [in this window] [in a new window] Table 1. Baseline Characteristics of Study Participants*

Geometric mean and median urinary concentrations of 11-dehydro thromboxane B_2 at baseline were significantly higher among patients who had subsequent development of the composite outcome of myocardial infarction, stroke, or cardiovascular death compared with those who remained free of these events (Table 2). The difference between cases and control subjects was greatest in those who had a myocardial infarction (24.5 versus 20.9 ng/mmol creatinine, $P=0.003$) or died of a cardiovascular cause (25.6 versus 20.4 ng/mmol creatinine, $P<0.001$). Baseline urinary concentrations of 11-dehydro thromboxane B_2 were not significantly different between cases who had subsequent development of stroke and their matched control group (25.0 versus 27.4 ng/mmol creatinine, $P=0.47$).

View this table: [in this window] [in a new window] Table 2. Baseline Urinary Concentrations of Urinary 11-Dehydro Thromboxane B_2 in Cases and Controls

The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of baseline urinary 11-dehydro thromboxane B_2 concentration (P for trend across quartiles, 0.01), with patients in the highest quartile having a risk 1.8-fold higher than those in the lowest quartile (OR, 1.8; 95% CI, 1.2 to 2.9; $P=0.009$) (Figure). A similar association was seen with myocardial infarction (P for trend across quartiles, 0.005) and cardiovascular death (P for trend across

quartiles, 0.001) but not for stroke (*P* for trend across quartiles, 0.20) (Table 3). Results were similar with or without adjustment for baseline differences between cases and control subjects, including conventional vascular risk factors, cointerventions, and randomized treatment allocation.



Association between quartiles of 11-dehydro thromboxane B_2 and composite of myocardial infarction (MI), stroke, or cardiovascular (CV) death after adjustment for baseline differences between cases and control subjects (*P* value is for trend of association).

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Table 3. Adjusted Odds* of Future Cardiovascular Death, Myocardial Infarction, and Stroke According to Baseline Urinary Concentrations of 11-Dehydro Thromboxane B_2

To evaluate whether increased baseline urinary 11-dehydro thromboxane B_2 concentrations were associated with early rather than late cardiovascular events, we performed separate analyses in patients who had an event within the first 12 months of study entry and those whose event occurred >12 months after study entry. The adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death that was associated with the highest quartile of urinary 11-dehydro thromboxane B_2 as compared with the lowest quartile was 2.9 (95% CI, 0.9 to 9.1) for events occurring with the first 12 months and 1.7 (95% CI, 1.0 to 2.7) for events occurring after the first 12 months.

Using linear multivariable regression modeling, variables that were found to be independently associated with baseline urinary 11-dehydro thromboxane B_2 concentrations in the urine were female sex (P=0.004), body mass index (P=0.001), history of peripheral vascular disease (P=0.01), current cigarette smoking (P=0.09), use of calcium channel blockers (P=0.08), and randomization

to vitamin E (P=0.04). However, these variables combined were able to predict <5% of the variation in urinary 11-dehydro thromboxane B_2 concentrations (R^2=0.045).

Discussion

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This is the first study to demonstrate an association between aspirin resistance, defined as failure of suppression of thromboxane generation, and cardiovascular risk. In a well-defined cohort of aspirin-treated patients at high risk of cardiovascular events, increasing baseline urinary concentrations of 11-dehydro thromboxane B_2 were associated with an increasing risk of cardiovascular events, particularly myocardial infarction and cardiovascular death. This association was strong, graded, and independent of conventional vascular risk factors, including elevated body mass index, blood pressure, hypertension, diabetes, smoking, and previous history of vascular disease. Moreover, the strength of the association was not modified by differences between cases and control subjects in the proportion of patients receiving lipid-lowering or antihypertensive therapy or by randomization to vitamin E or ACE inhibitors.

Several mechanisms can be proposed to account for the incomplete suppression of thromboxane generation by aspirin.[2] First, polymorphisms or mutations of the cyclooxygenase-1 gene that make it relatively resistant to inhibition by aspirin may provide a molecular basis for aspirin resistance. However, to our knowledge, such a mutation has not been identified. Second, nucleated cells such as monocytes or vascular endothelial cells can provide prostaglandin H_2 to platelets to bypass platelet cyclooxygenase-1 or can use prostaglandin H_2 to synthesize their own thromboxane A_2 because they are endowed with substantial amounts of thromboxane synthase.[4,18–20] Arachidonate conversion to prostaglandin H_2 is catalyzed by cyclooxygenase-1 or -2. Although low-dose aspirin permanently and completely blocks cyclooxygenase-1 in platelets, nucleated cells can regenerate the enzyme. Consequently, these cells can produce prostaglandin H_2 even in the face of aspirin treatment. In addition to cyclooxygenase-1–mediated prostaglandin H_2 generation, nucleated cells can also produce prostaglandin H_2 through cyclooxygenase-2.[20,21] Whereas cyclooxygenase-1 is blocked by 80 to 325 mg of aspirin, doses similar to that used in the HOPE trial, inhibition of cyclooxygenase-2 requires doses of

aspirin in excess of 500 mg daily.[2] Unlike cyclooxygenase-1, which is constitutively expressed, cyclooxygenase-2 expression in nucleated cells is augmented 10- to 20-fold by inflammatory stimuli. Augmented cyclooxygenase-2 expression may contribute to aspirin resistance in patients with ischemic heart disease because atherosclerosis is an inflammatory disease.[21-23]

Our finding of an independent albeit weak association between history of peripheral vascular disease and urinary 11-dehydro thromboxane B_2 levels in the urine is consistent with prior reports suggesting that the severity of atherosclerosis is an important determinant of thromboxane generation.[14,24] In patients being treated with aspirin, differences in the extent or severity of atherosclerosis are unlikely to affect de novo platelet thromboxane production because even very low doses of aspirin completely and irreversibly block platelet cyclooxygenase-1.[25] Upregulation of cyclooxygenase-2 has been demonstrated in atherosclerotic tissue[24] and may be associated with greater synthesis and transfer of prostaglandin H_2 to platelets, thereby bypassing platelet cyclooxygenase-1 and leading to aspirin-insensitive thromboxane biosynthesis in these patients. However, our study cannot distinguish between failure of suppression of platelet cyclooxygenase-1 and upregulation of COX-2 expression as the cause for the observed differences in 11-dehydro thromboxane B_2 excretion between cases and control subjects.

The reason for the lack of an association between urinary 11-dehydro thromboxane B_2 and risk of stroke is unclear. Aspirin reduces the risk of stroke in a broad category of high-risk patients[1,2]; elevated urinary concentrations of 11-dehydro thromboxane B_2 have been reported in patients after stroke,[26] and failure of aspirin to suppress "platelet reactivity" or inhibit platelet aggregation in response to various platelet agonists also has been documented in patients after stroke.[27-29] In our study, the mean urinary concentration of 11-dehydro thromboxane B_2 in cases who had a stroke was similar to that in all cases (25.0 versus 24.5 ng/mmol creatinine), but the corresponding urinary concentration in matched stroke control subjects with was higher than the concentration in all control subjects (27.4 versus 21.5 ng/mmol creatinine). However, the number of stroke cases and matched control subjects was relatively small (n=80). Given the clear and graded association between urinary 11-dehydro thromboxane B_2 concentration and the composite outcome of myocardial infarction, stroke, or cardiovascular death, as well as other individual components of this outcome, the absence of a demonstrable association between stroke risk and urinary concentration of 11-dehydro

thromboxane B_2 probably reflects a play of chance.

Our study has several potential limitations. First, there were important differences between cases and control subjects with regard to potentially important confounders, including body mass index, systolic blood pressure, hypertension, diabetes, smoking, history of vascular disease, and cointerventions. However, even after adjustment for these differences, a clear association between urinary 11-dehydro thromboxane B_2 concentrations and risk of death, myocardial infarction, and stroke was demonstrated. The weak association between baseline patient characteristics and urinary 11-dehydro thromboxane B_2 concentrations further supports the conclusion that confounding did not account for our results. Second, urinary 11-dehydro thromboxane B_2 concentrations may have been influenced by recent acute thrombotic events, such as myocardial infarction or stroke, processes that are known to be associated with platelet activation and enhanced urinary excretion of thromboxane metabolites. However, patients who had a myocardial infarction or stroke within the previous 7 weeks were not randomized into the HOPE study, making this explanation less likely. Third, single baseline determinations of urinary 11-dehydro thromboxane B_2 concentrations may not accurately reflect the extent of platelet activation over long periods of time. However, the association between elevated urinary 11-dehydro thromboxane B_2 concentrations at baseline and subsequent risk of cardiovascular events was evident both during the first 12 months after randomization and beyond 12 months, indicating a stable association over an extended period of time. Fourth, we did not confirm patient compliance with aspirin therapy by measuring salicylate levels in the blood or urine. However, we specifically assessed compliance with aspirin therapy at each follow-up visit and only considered patients for inclusion who were taking aspirin before randomization and at 6-month follow-up visits. Patients who discontinued aspirin at any time during the study were not included. Finally, the extent of biological variation in urinary 11-dehydro thromboxane B_2 levels is unknown but could potentially limit the value of this marker to predict the risk of future cardiovascular events in an individual patient.

We conclude that among aspirin-treated patients at high risk of cardiovascular events, persistent thromboxane generation predicts the risk of the composite outcome of myocardial infarction, stroke, or cardiovascular death, independent of other cardiovascular risk factors. These data raise the possibility that high urinary levels of 11-dehydro thromboxane B_2 can prospectively identify

patients who are relatively resistant to conventional antithrombotic doses of aspirin and who may benefit from additional antiplatelet therapies or treatments that more effectively block thromboxane production or activity.

Acknowledgments

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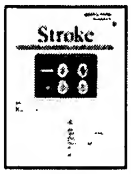

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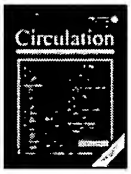

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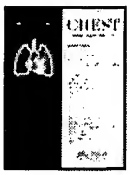

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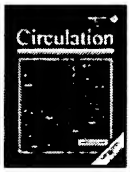

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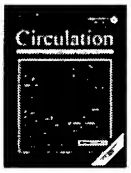

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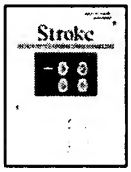

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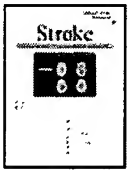

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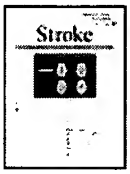

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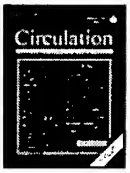

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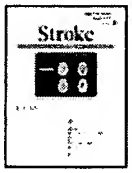

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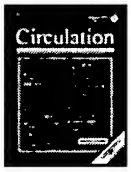

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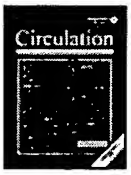

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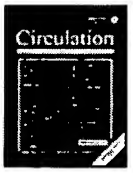

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Technology to determine a patient's response to aspirin therapy taken to prevent heart attacks

Devices/Technology
Published: Monday, 8-Nov-2004

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Corgenix Medical Corporation and AspirinWorks, today announced that together they have entered into a license agreement with McMaster University, Hamilton, Ontario, providing Corgenix and CCC exclusive rights to the propriety technology owned by McMaster for the development, manufacturing and marketing of innovative diagnostic tests specific to the pathway by which aspirin acts on platelets.

This technology has demonstrated the ability to assess an individual's relative risk for heart attack by measuring the person's degree of aspirin resistance.

The technology involves the measurement of a unique thromboxane metabolite, which removes the guesswork, allowing physicians to quantify the amount of the metabolite involved in aspirin resistance. Qualitative platelet function tests currently available are subject to multiple interferences. Once a physician measures a patient's response to aspirin, the dosage can be adjusted or alternative platelet therapy recommended.

Mamdouh Shoukri, Vice-President (Research and International Affairs) at McMaster University, said, "McMaster has long been committed not only to develop technology beneficial to the healthcare system, but to collaborate with outstanding commercial partners like Corgenix and AspirinWorks to ensure that our technology actually gets to the public. This technology for aspirin resistance, developed in our Michael G. DeGroote School of Medicine, is a ground-breaking discovery in the prevention of cardiovascular disease."

"Part of our mission as an academic teaching hospital is to advance health care through education and research," said Bill MacLeod, Vice-President, Research and Corporate Development at Hamilton Health Sciences. "This collaboration with our academic partner, McMaster University, is another

example of how we are working together to move important clinical research out of the lab to make a difference to people and their health."

Jack Hirsh, MD, Professor Emeritus of Medicine at the Michael G. DeGroote School of Medicine at McMaster, one of the co-developers of the aspirin resistance technology, co-authored a 2002 study which demonstrated that patients taking aspirin with high levels of thromboxane in their urine had a risk of cardiovascular-related death that was 3.5 times as great as those on aspirin with the expected low therapeutic levels. "While medications to lower hypertension and cholesterol are tested through measurement of blood pressure and blood cholesterol levels, aspirin has not been routinely monitored to see if it is truly protecting a patient against heart attack or stroke," said Dr. Hirsh. "Patients should be tested for aspirin resistance so we can determine if the aspirin is working and if it's not, increase the dosage and retest, or choose another anti-platelet therapy."

Atherothrombosis is the leading cause of death worldwide, accounting for 52% of all deaths. An estimated 56 million people worldwide died from atherothrombotic disease in 2000 (manifested as cardiovascular disease, ischemic heart disease and cerebrovascular disease). Atherothrombosis is also the leading cause of death in the US, in 1999 responsible for almost 1 million deaths and a contributing factor in 70% of all deaths. According to the most recent Centers for Disease Control computations, the probability at birth of eventually dying from major cardiovascular diseases is 47 percent, whereas the chance of dying from cancer is 22 percent.

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